Appendix 2

Pharmatox

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IN VITRO EYE IRRITATION

of Emulsion B8575

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1. SUMMARY

The EYTEX method is an *in vitro* test used to predict ocular irritation, based on the alterations in a protein matrix. The potential of Emulsion B8575 for ocular irritation was investigated in the Eytex Upright Membrane Assay (UMA).

The sample was found to be a non-irritant to minimal irritant.

2. INTRODUCTION

2.1. Sponsor

EGO Pharmaceuticals Pty. Ltd.

2.2. Project number

Project **T1716.1.A**

2.3. Sample Description

Emulsion B8575, a white cream stored at room temperature (between 19 and 24° C).
The reactivity and physical data were determined by EGO Pharmaceuticals Pty. Ltd.

2.4. Rationale of the study

The Eytex system has been evaluated by using more than one hundred products. The results were compared with *in vivo* data obtained previously for each chemical and the European Economic Cooperation (EEC) labelling for dangerous substances (Regnier and Imbert, 1990). The coefficient of the linear correlation between EYTEX score and Draize score was 0.85. Specificity (ratio of "non-Irritants" *in vivo* giving negative results to the total) was 90.5% (48/53). Sensitivity (ratio of "Irritants" *in vivo* giving positive responses to the total) was 91.3% (42/46). The predictive value for identifying "irritants" (moderately to extremely irritating products) and "non-irritants" (non- to mildly irritating products) were 89.3% (42/47) and 92.3% (48/52), respectively. Based on the EEC guidelines for classification and labelling of dangerous substances, 38 of 43 (88.3%) products labelled as irritant or severely irritant were correctly classified as irritants or severely irritants and 47 of 53 (88.6%) non-irritants were correctly classified as non-irritants by the EYTEX system.

Based on these results, the EYTEX is considered method as a valuable tool for predicting eye irritancy.

Considering that the majority of eye irritating products cause a corneal opacity, that the major mechanism of corneal opacification is the denaturation and/or the precipitation of corneal proteins (Rozen, 1972) and 72 % (80/110) of Draize score depends on effects on cornea (Draize *et al*, 1944), it appears logical to think that a test which would mimic the corneal opacification could be predictive of occular irritancy. Recently, an *in vitro* method for the evaluation of ocular irritation-EYTEX- has been developed

(Gordon and Kelly, 1989a; 1989b; Gordon *et al*, 1989; 1990). The EYTEX system is based on aggregation of the protein reagent caused by known concentration of well-characterised eye irritants, and provides an approximation of maximum acute toxic response.

In addition, the animal care and ethics commitee does not approve the carrying out of the Draize test as required by law under Schedule1 (4)(b) Animal Research (Amendment) 1989 to the New South Wales animal research act 1985, Australia.

2.5. Object

To determine the potential for ocular irritation caused by the test substance using the EYTEX™ method which is an *in vitro* test, based on the alterations of a protein matrix..

2.6. Basic principle

The EYTEX Reagent is a protein reagent (globulins, albumin, muccopolysaccharides and lipids along with buffer salts). It is reconstituted by the addition of distilled water. The EYTEX system undergoes a process of denaturation when challenged with a chemical irritant. Protein denaturation has been identified as the major component of corneal injury and ocular irritation. At the endpoint of the assay, opacification of the reagent is measured on a colorimeter. Calibrators provide a direct comparison to the Draize Scale to determine Ocular Safety Classifications.

2.7. Protocol selection and procedural summary

The samples are analysed by direct application to the barrier matrix and incubation of the matrix in contact with the reagent for a period of 24 hours by using the Upright Membrane Assay.

Calibrators and Controls

The system is calibrated by the use of three well-characterised eye-irritants and two Quality Control Samples are analysed in each assay to ensure standardization.

Qualification

The protocol has qualification steps which must be completed and fulfilled for a result to be accepted. Classification and determination of the EYTEX/Draize Equivalent Irritation Index is then carried out.

3. RESULTS

The Eytex/Draize equivalence score for Emulsion B8575 was 2.4.
The dose response curve corresponds to a 24 hour reading and can be seen in the Appendix.

4. CONCLUSION

An Eytex Upright Membrane Assay (UMA) was carried out on a sample of Emulsion B8575.

The sample was found to be a non-irritant to minimal irritant.

References

Regnier J-F and Imbert C, Validation on the EYTEX system as a screen for predicting the ocular irritancy potential of chemical products, *internal report*, 1990.

Draize JH, Woodward G, and Calvery HO, Methods for the study of irritation of toxicity of substances applied topically to the skin and mucous membrane, *J Pharmacol Exp Therap* **82**: 377-390, 1944.

Rozen MJ, The relationship of structure to properties in surfactants, *J Am Oil Chem Soc* **49**: 293-297, 1972.

Gordon VC and Kelly CP, An *in vitro* method for determining ocular irritation, *Cosmetics & Toiletries* 104/10,69, 1989a.

Gordon VC and Kelly CP, Validation of an *in vitro* method for determining ocular irritation of cosmetic ingredients and products,
Cosmetics & Toiletries 104/11,67, 1989b.

Gordon VC, Kelly CP, and Bergman HC, Application of the EYTEX method, Second international conference on practical *in vitro* toxicology, Nottingham, UK, July:22-47, 1989.

Gordon VC, Kelly CP, and Bergman HC, The Eytex system, scientific validation and applications of the EYTEX system to the *in vitro* prediction of ocular irritation, presented to the EEC scientific commission, Brussels, Belgium, *unpublished data*, 1990.

Appendix

- Dose Response Curve
- Equivalence Table

Sample: Emulsion B8575
Company: EGO Pharmaceuticals Pty. Ltd.
Code: T1716.1.A
Protocol: UMA/Eytex
Date: 10/03/1995

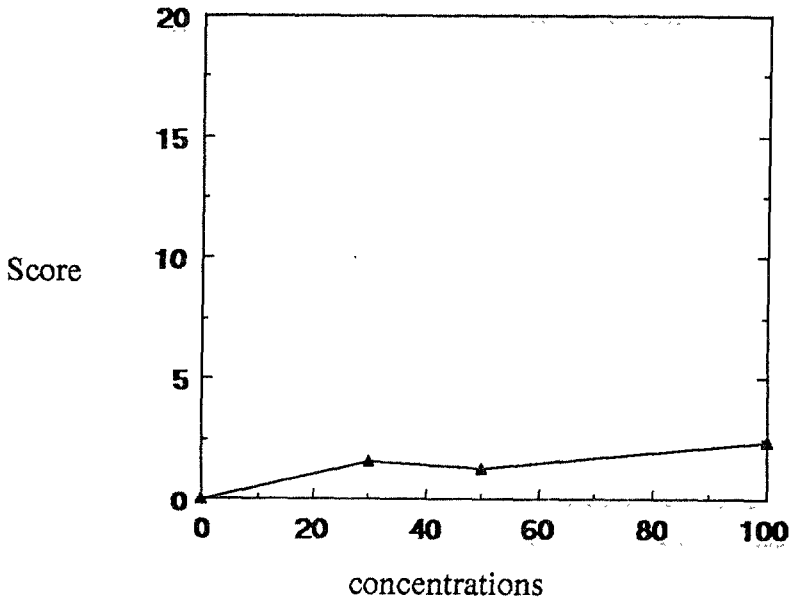
Sample Result: Volume: 100 µl Classification: Non-irritant to minimal

EDE EQUIVALENT: 2.36

Cuvette	Sample OD	Blank OD	Net OD	Score	Classification
CRO	135				
CR1	122				
CR2	586				
CR3	1720				
100 µl	26	3	23	2.36	NI/Min
50 µl	18	5	13	1.33	NI/Min
30µl	22	7	15	1.54	NI/Min

Senior Technician: F.Brook

Dose response curve



EYTEX/DRAIZE EQUIVALENCE - UMA PROTOCOL

The Eytex/Draize Equivalent (EDE) is calculated from the calibration curve of irritants with known *in vivo* Draize results.

The Eytex scoring diagram classified the EDE for the UMA protocol as follows:

Minimal	0 - 10.6
Min/Mild	10.7 - 24.5
Mild	24.6 - 28.8
Mild/Mod	28.9 - 31.4
Moderate	31.5 - 39.9
Mod/Sev	40 - 51
Severe	≥ 52

All test samples with *in vitro* Draize equivalents which are ≥ 24.5 are positive results or potential irritants.